

Figure 1

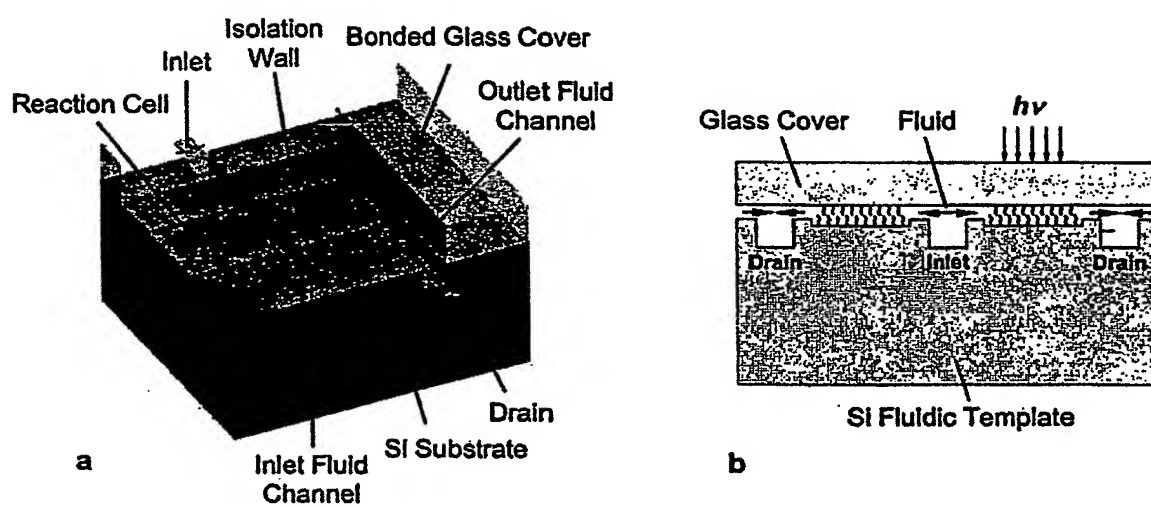


Figure 2

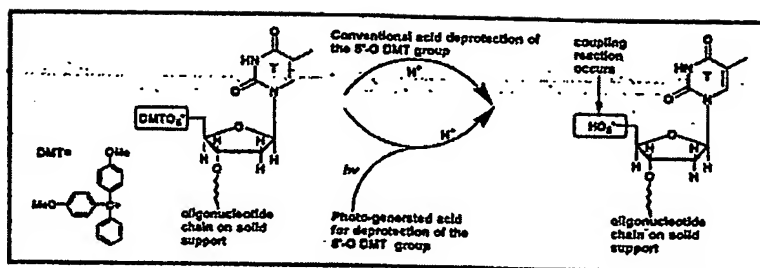


Figure 3

10/533208

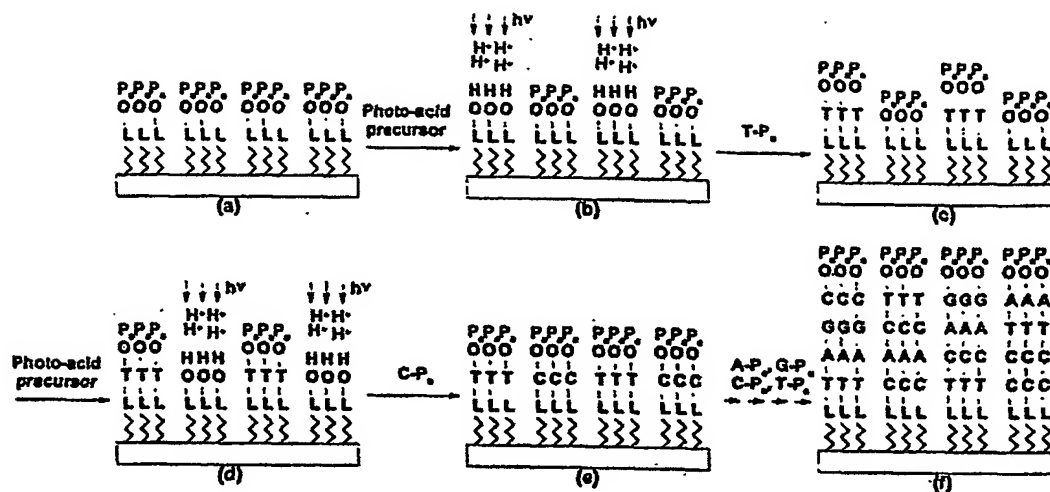


Figure 4

10/533208

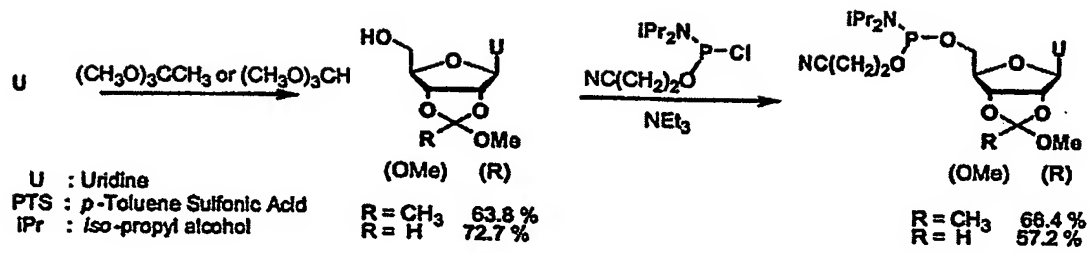


Figure 5

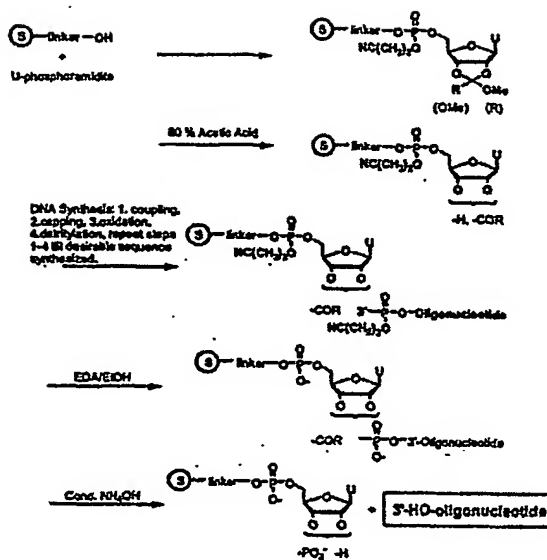


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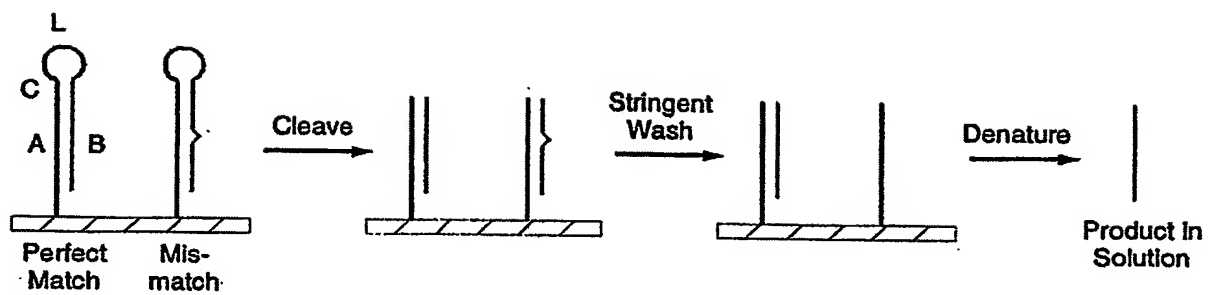


Figure 7

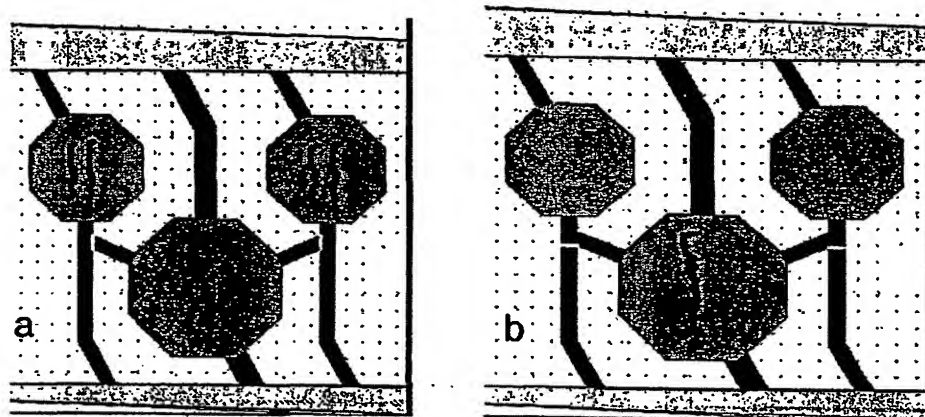


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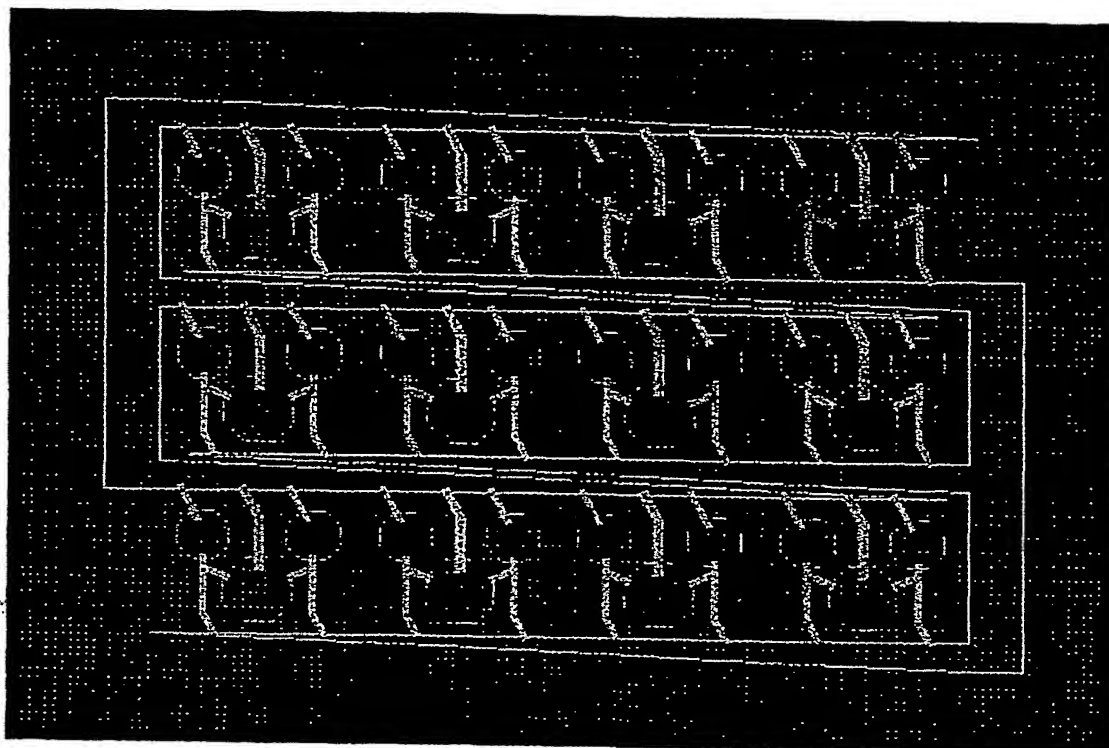
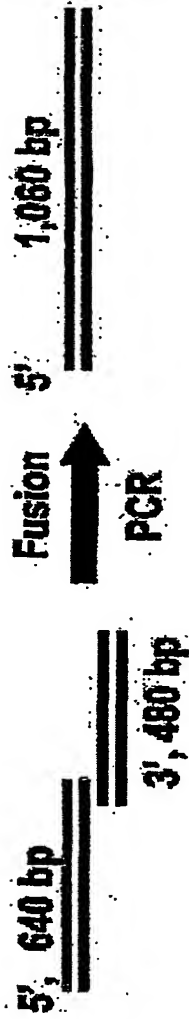


Figure 9

Fusion PCR for Multi-Stage Long Gene Assembling



pol A		pol B
2.0	2.5	1.5 2.0 mM Mg
0.25 5.75	0.25 5.75	0.6 % DMSO

5', 640 bp		3', 480 bp	
T1	T2	T1	T2
10	1	10	1



Figure 10

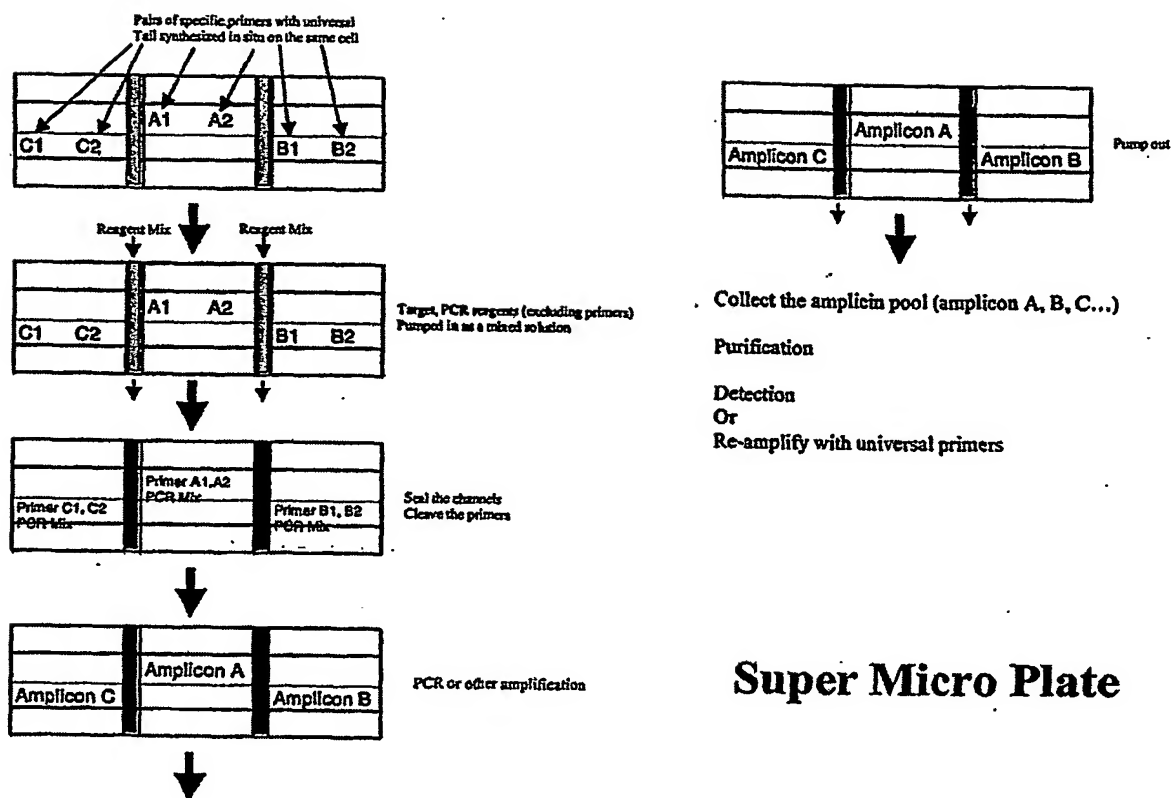


Figure 11

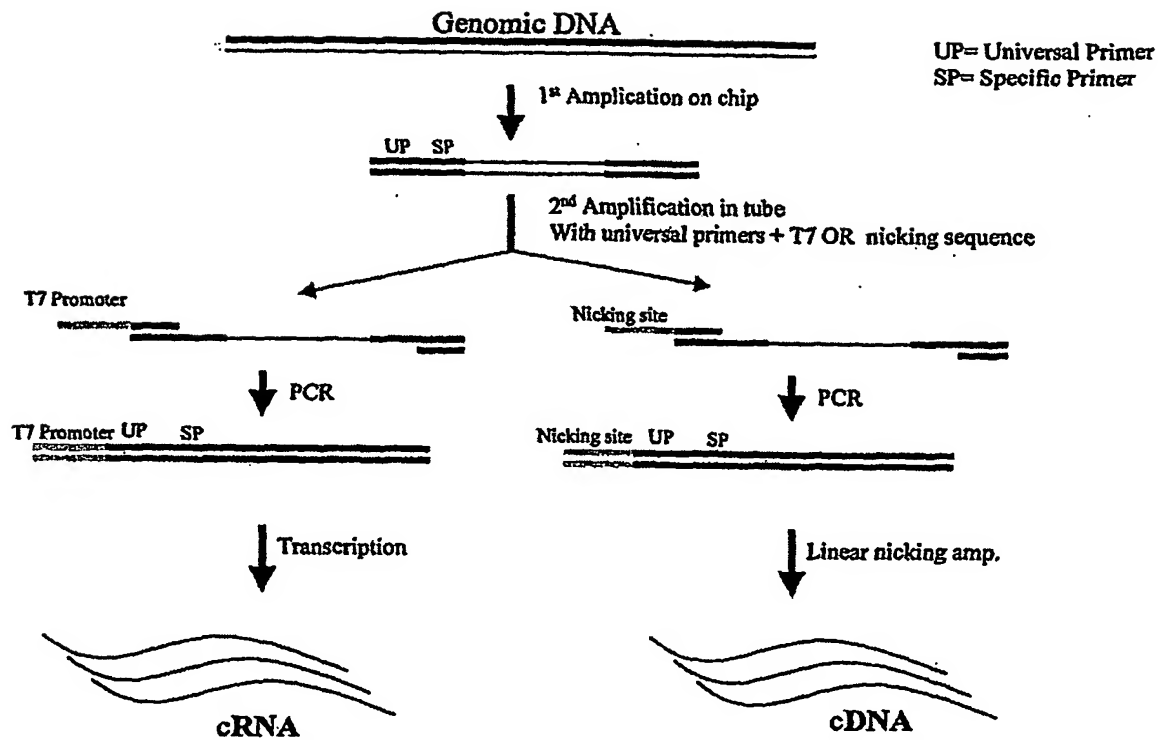


Figure 12

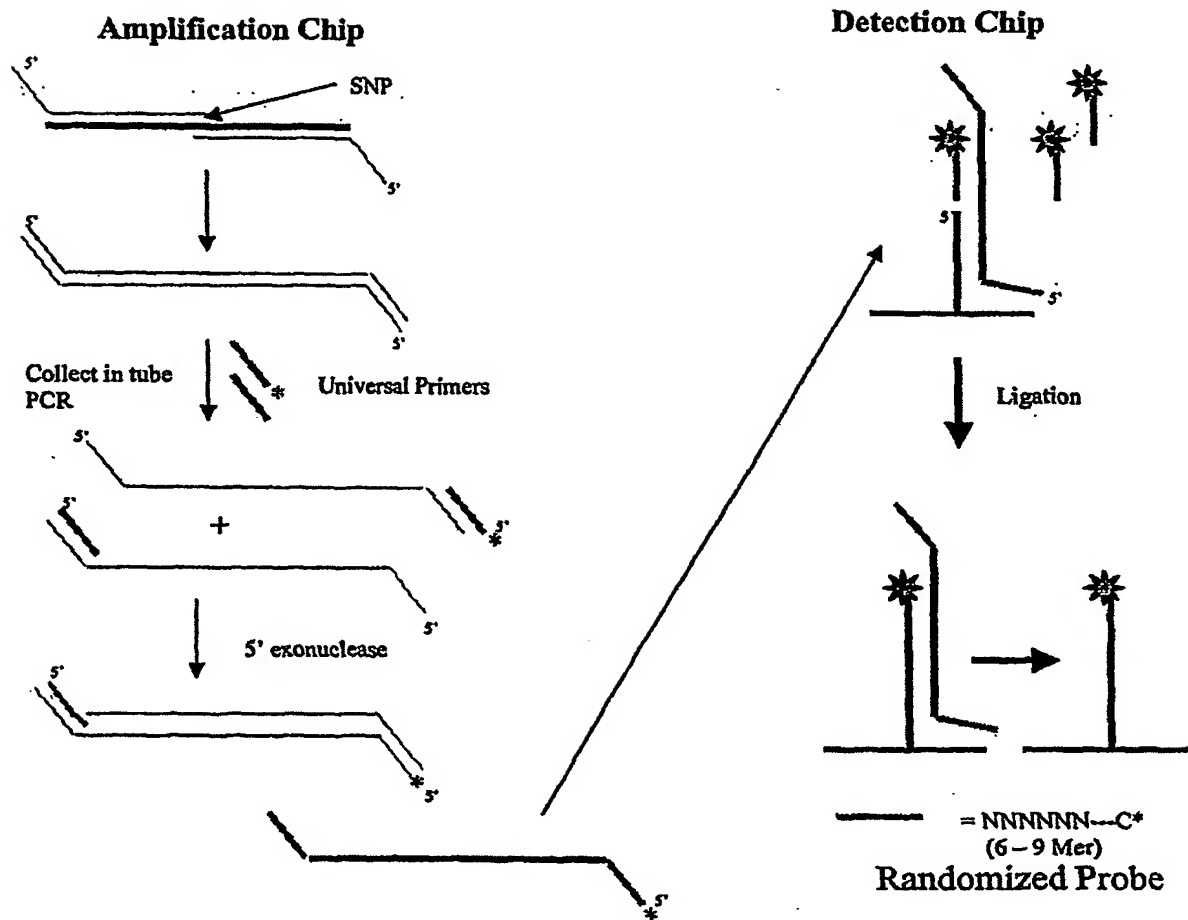


Figure 13

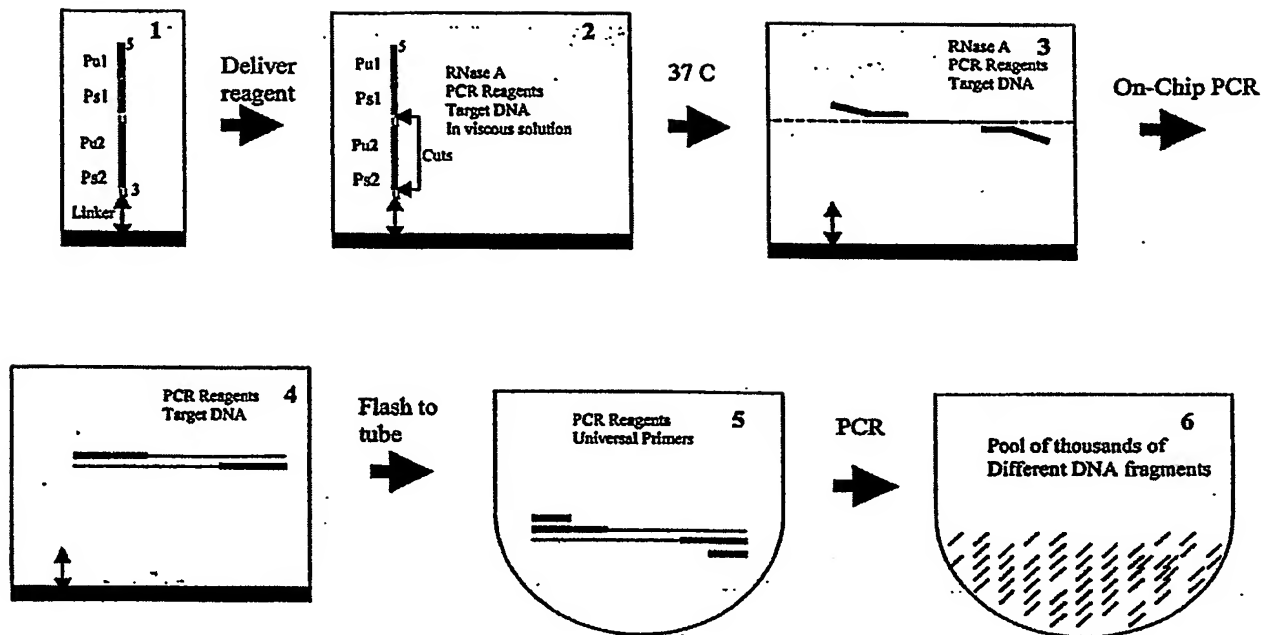
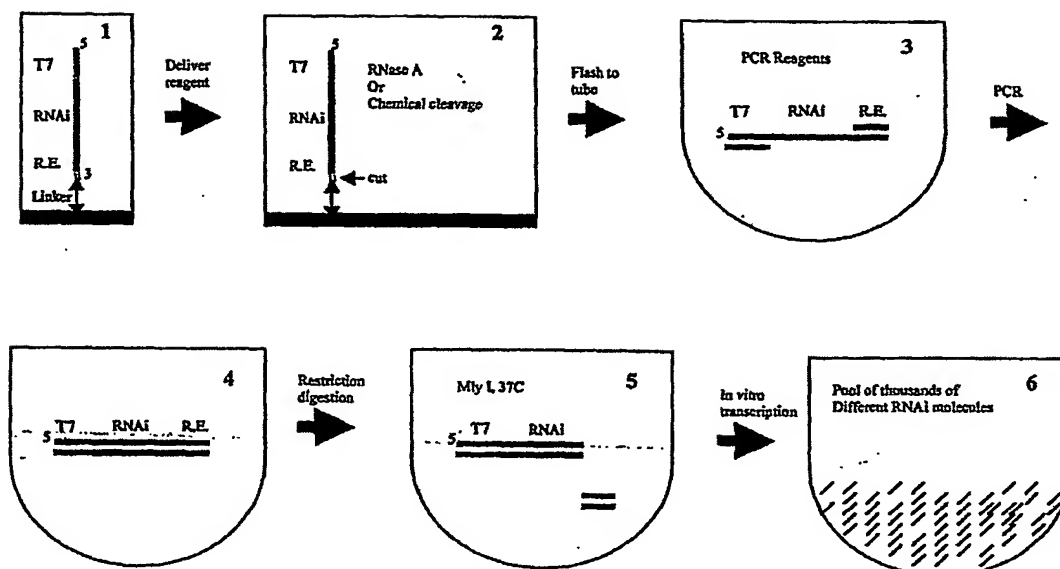


Figure 14



1. In situ synthesized oligos (about 60mer). Each oligo contains a rU, one T7 promoter, specific RNAi sequence and a Mly I restriction site. Mly I is a blunt cutting enzyme (GAGTCNNNNN⁺).
2. Oligos are cleaved by RNase A or chemicals.
3. Flash the oligos into tube. PCR amplify the targets with universal primers, one contains the T7 sequence and the other contains the restriction sequence.
4. Double stranded PCR products with thousands of specific RNAi sequences and common T7 and restriction site.
5. Digest the PCR products with Mly I enzyme to remove the tail.
6. In vitro transcription to produce unlimited supply of RNAi pools (can be re-amplified and re-transcribed) ready for use.

Figure 15

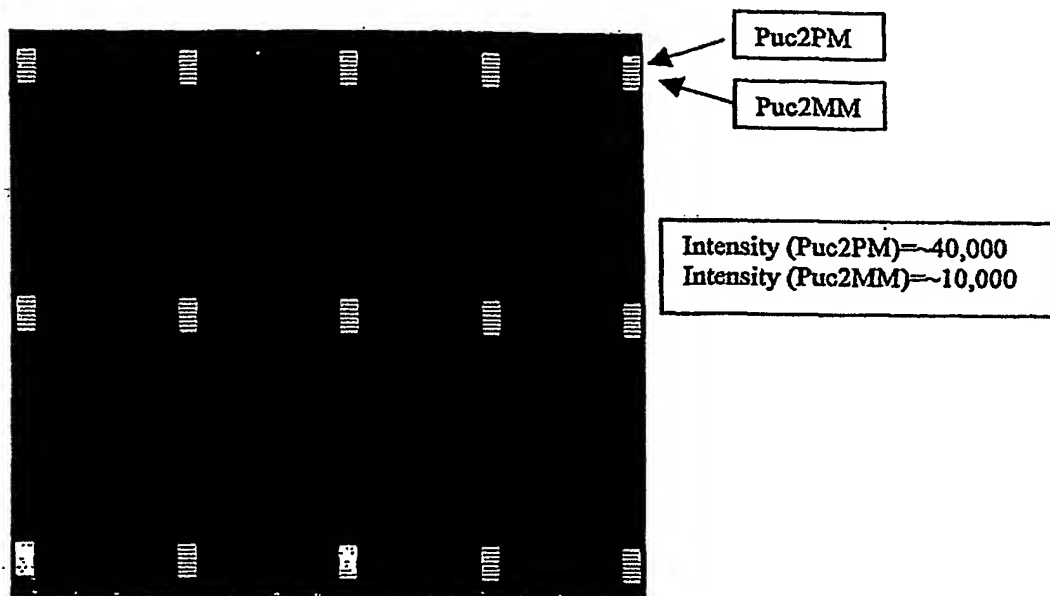


Figure 16

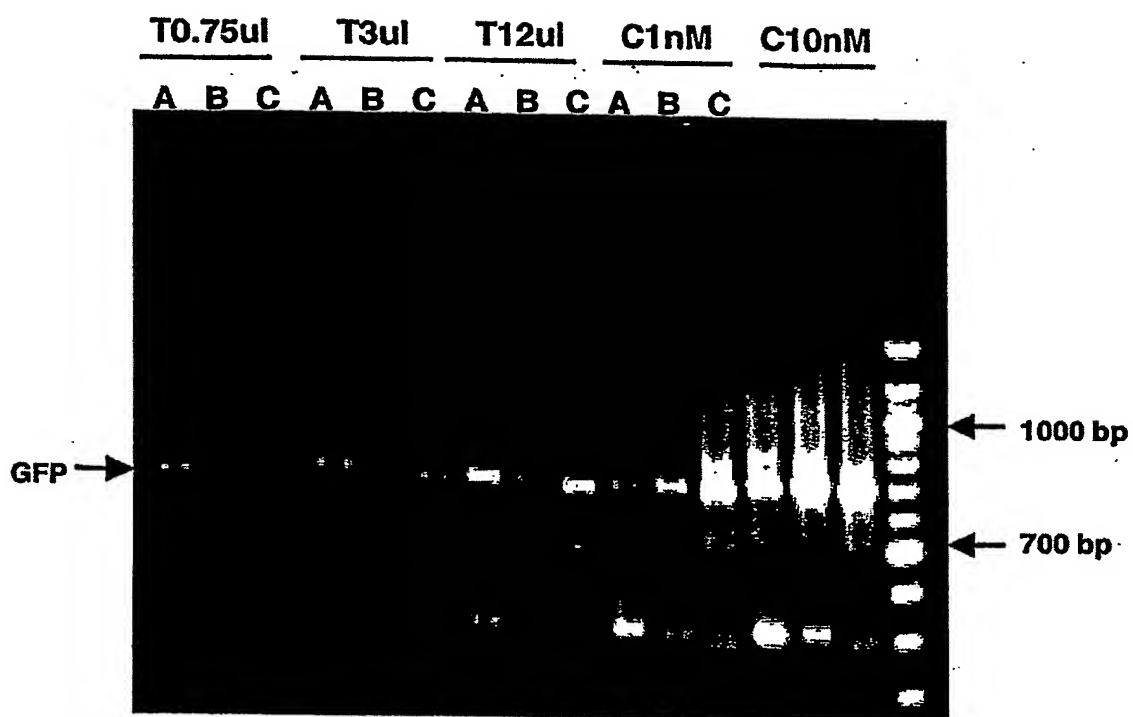


Figure 17

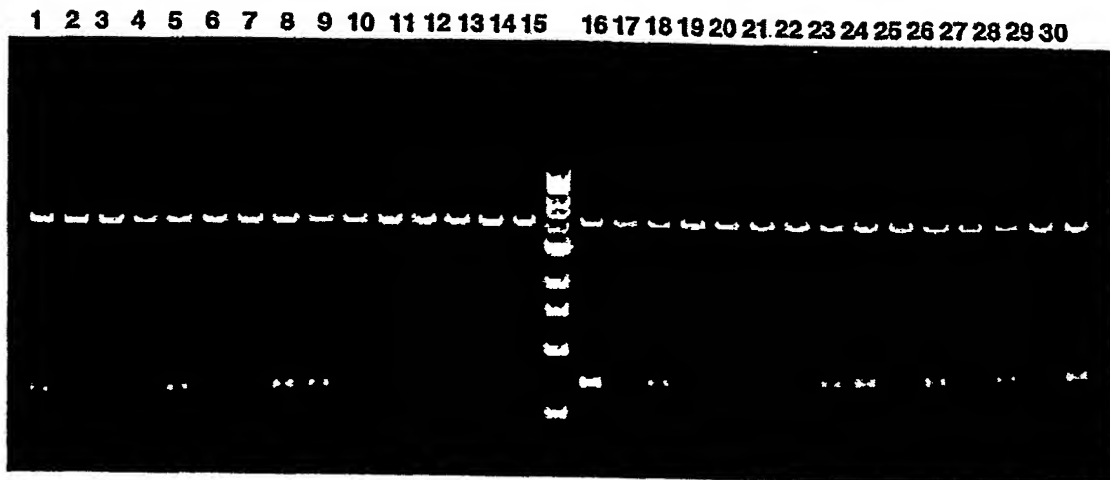
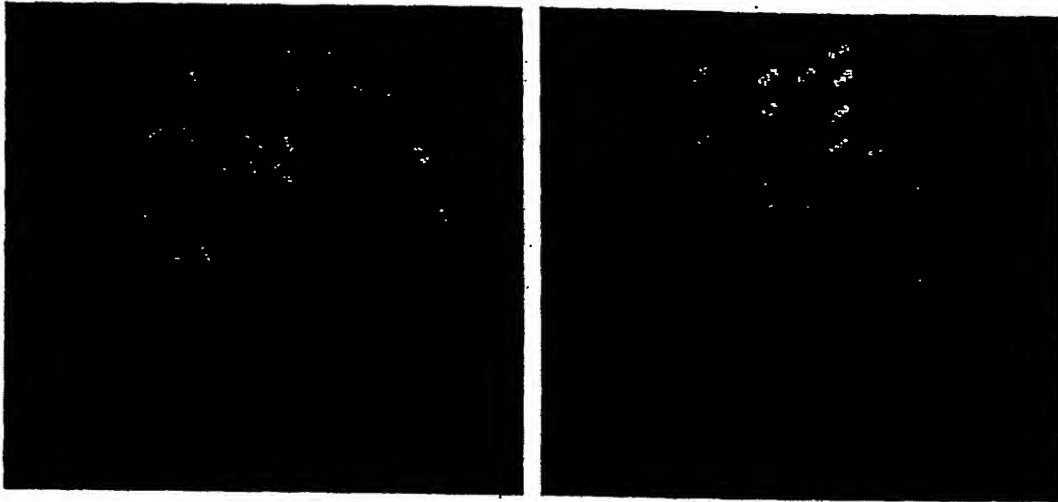


Figure 18



Dish 1

Dish 2

Figure 19



Figure 20

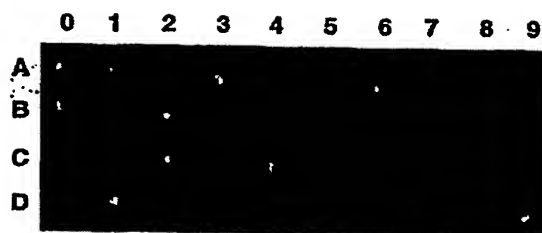


Figure 21

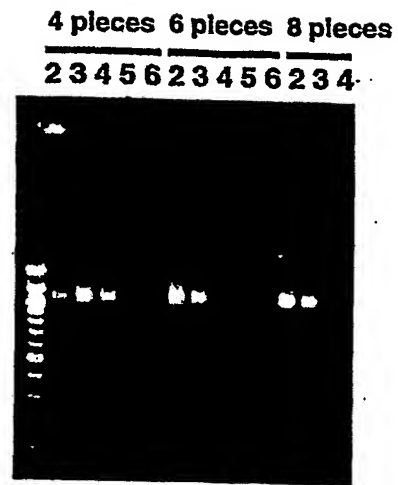


Figure 22

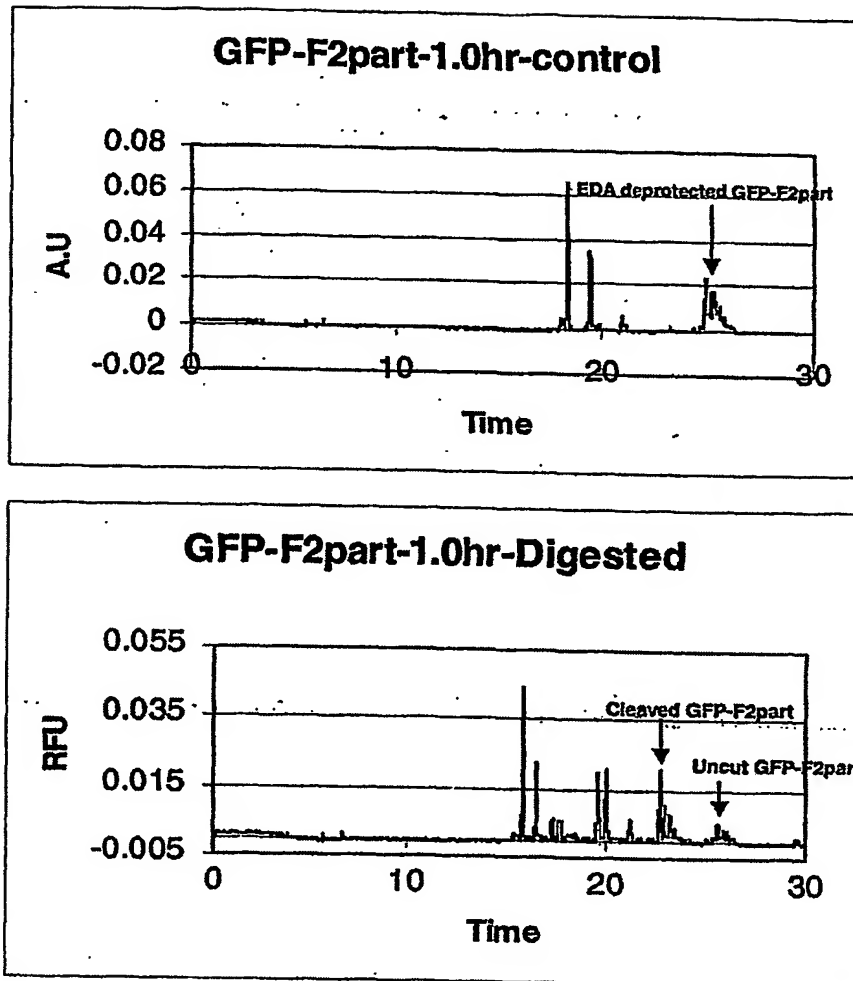


Figure 23

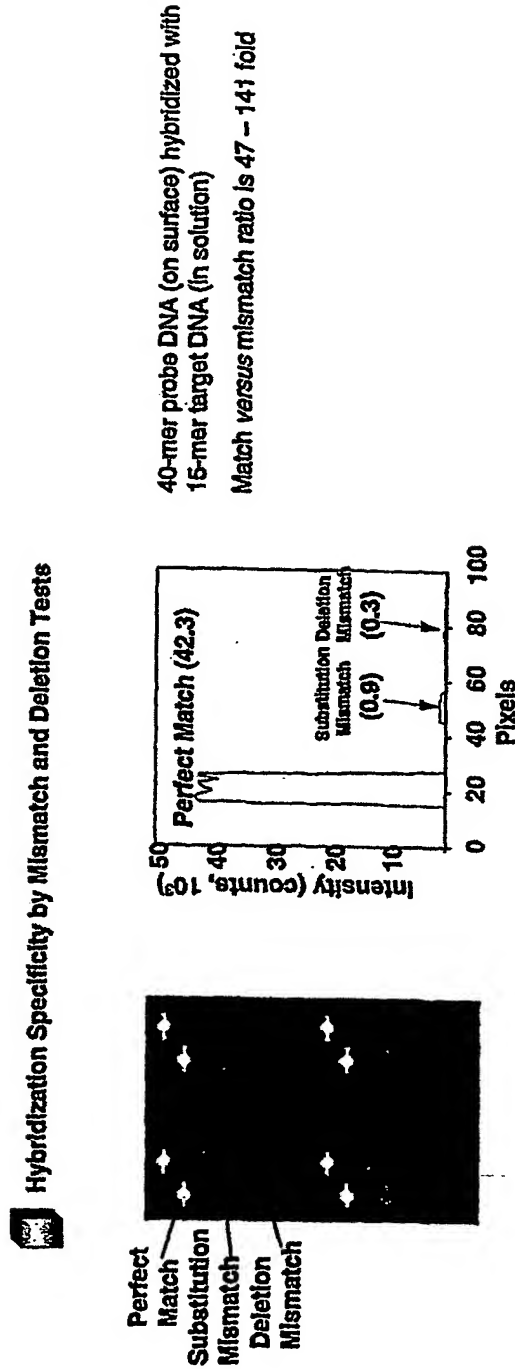


Figure 24

Synthesis of DNA Oligos Greater than 100 bases

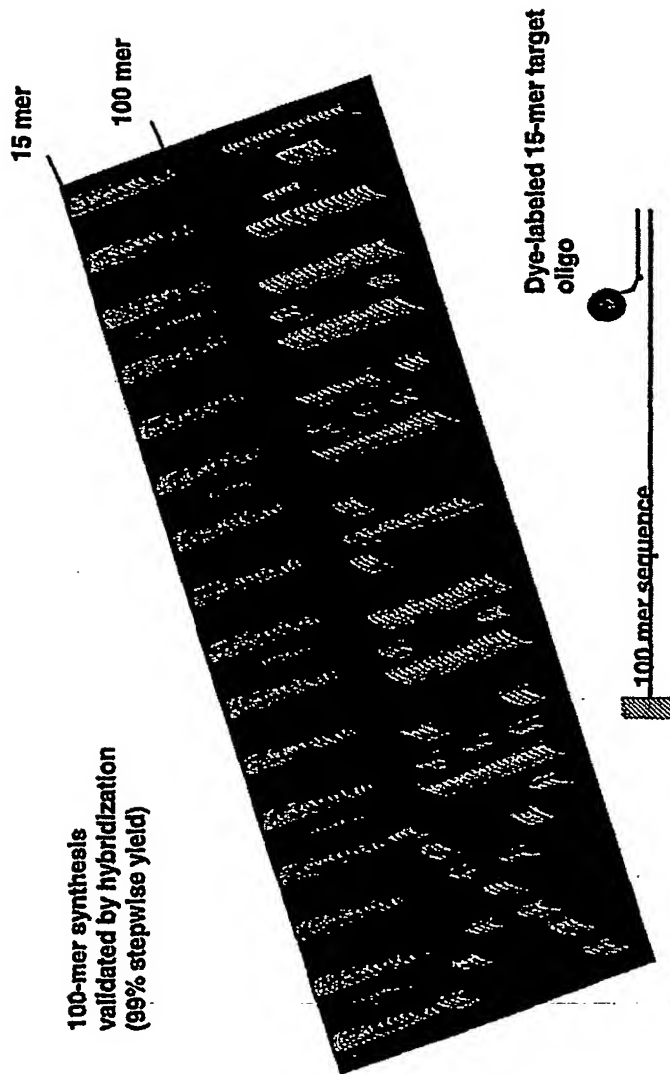


Figure 25

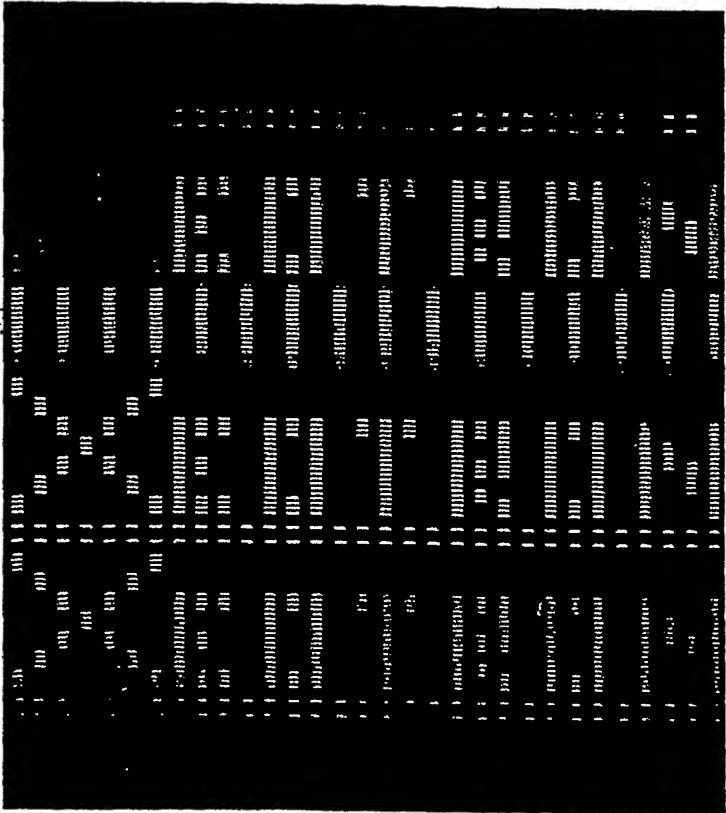


Figure 26

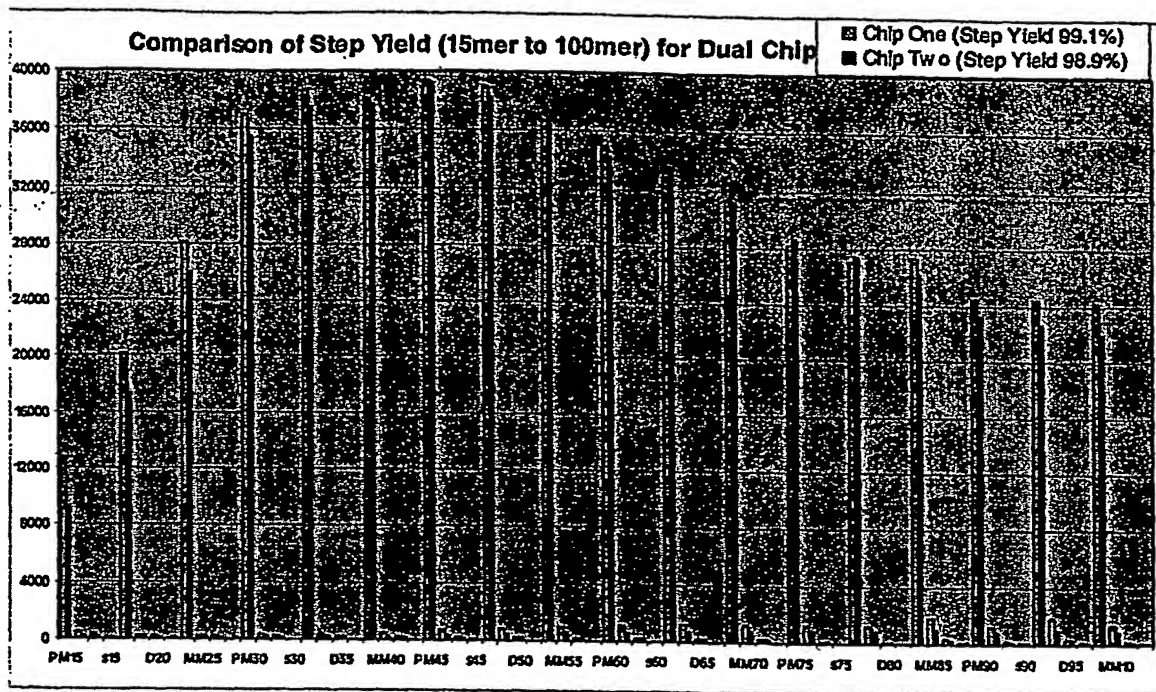


Figure 27

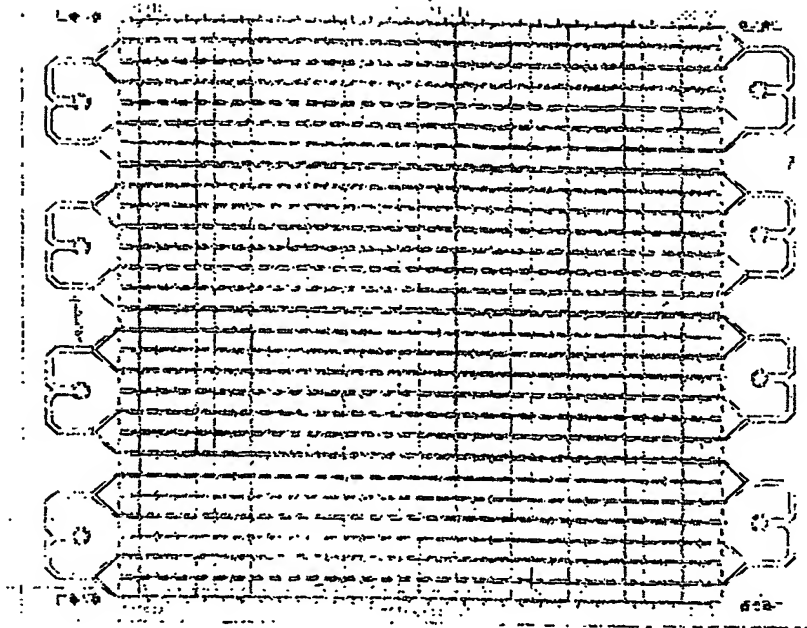


Figure 28

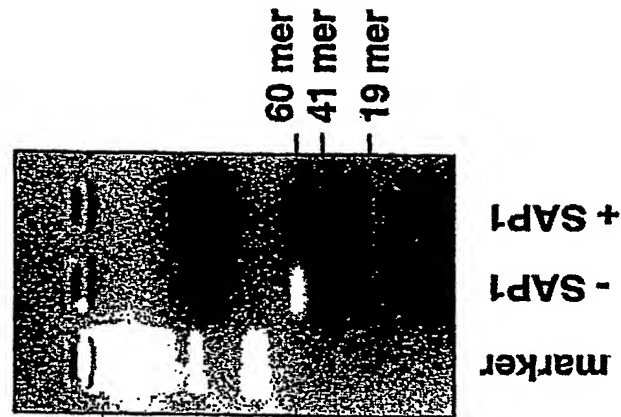
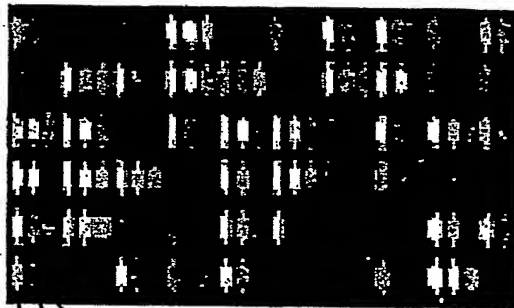


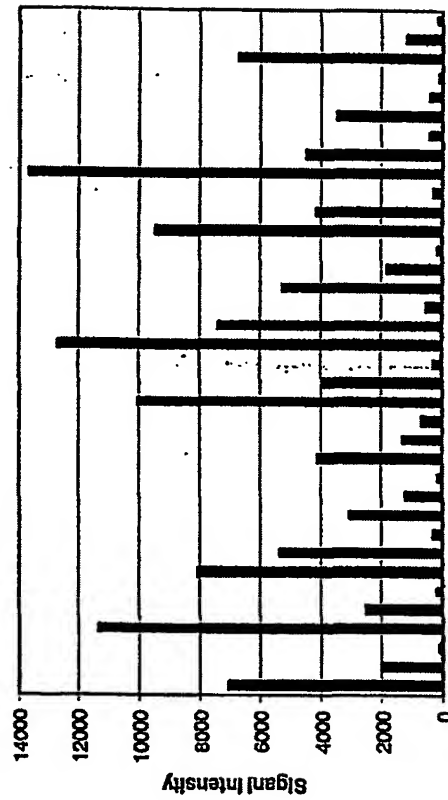
Figure 29

Electrophoresis
gel analysis of
PCR sequences
(60 mers) and the
sequences after
SAP1 enzyme
digestion (41 and
19 mers)



Perfect
Match
Single Deletion
Double Deletion

A



B

Probes
Figure 30

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